Lipase(LPS) Assay Kit (Spectrophotometry)

Product Description

Lipase (LPS), also known as triacylglycerol acylhydrolase, catalyzes the hydrolysis of triglycerides to produce fatty acids and glycerol (or diglycerides and monoglycerides). LPS is widely distributed in various organisms. An abnormal increase of LPS in serum is commonly seen in pancreatitis and pancreatic cancer.

Assay Principle

LPS catalyzes the hydrolysis of oil esters into fatty acids. The activity of LPS is determined by measuring the generation rate of fatty acids using the copper soap method.

Kit Components

Taking 50T/48S packing for example:

Catalog No.	Specification	Storage
CB0092S - A	80mL x 1 vial	4℃
CB0092S - B	10mL x 1 vial	Store at room temperature.
CB0092S - C	20mL x 1 vial	4℃
CB0092S - Standard	Powder x 1 vial	Store at 4° C. Prior to use, reconstitute the standard by adding 1.5 mL of anhydrous ethanol to prepare a 125 μmol/mL oleic acid standard stock solution, and dissolve fully. Thaw and dissolve completely before use.

Note: It is highly recommended to select 2–3 samples with large variations for a preliminary pilot assay before performing the formal measurement.

Instruction for Use

I. Preparation of Lab Instruments

Mortar and pestle, benchtop centrifuge, vortex mixer/shaker, visible spectrophotometer, 1mL glass cuvettes, adjustable pipettes, toluene, anhydrous ethanol, ice, and distilled water.

II. Crude Enzyme Extraction

1. Serum or Liquid Samples: Detect directly.
2. Tissue Samples: Weigh 100 mg of tissue, add 1 mL of CB0092S-A and homogenize in an ice bath. Centrifuge at 15000 rpm at 4°C for 30 min, collect the supernatant, and keep on ice for assaying.

III. Measurement Steps

1. Preheat the spectrophotometer for at least 30 min, set the wavelength to 710 nm, and zero the instrument using tuluene.
2. Preheat CB0092S-A and CB0092S-B in a 37°C water bath for 30 min.
3. Dilution of Standard Solutions: Dilute the 125 μmol/mL oleic acid standard stock solution with anhydrous ethanol to obtain a series of standard solutions: 125, 62.5, 31.25, 15.625, 7.8125, and 3.9 μmol/mL.

4. Operation Table:

Add the following reagents sequentially into the centrifuge tubes:

Reagent	Blank Tube (μL)	Sample Tube (μL)	Standard Tube (μL)
CB0092S - A	375	375	375
CB0092S - B	125	125	125
Vortex and mix repeatedly			
Distilled Water	200	-	-
Supernatant / Serum	-	200	-
Standard Solution	-	-	200
Vortex and mix quickly, then incubate in a 37°C water bath for exactly 10 min			
Toluene	1000	1000	1000
Vortex and mix repeatedly, then centrifuge at 4000 rpm at room temperature for 10 min.			
Take out the centrifuge tubes, carefully aspirate 0.9 mL of the upper layer solution, and transfer it into another 2 mL plastic centrifuge tube. Then operate according to the following table:			
CB0092S - C	225	225	225
Vortex and mix repeatedly; centrifuge at 4000 rpm at room temperature for 10 min. Carefully aspirate 800μL of the upper layer solution, transfer it into a 1mL glass cuvette, and measure the absorbance at 710 nm. Record the absorbance values as Ablk, Asp, and Astd. Calculate: ΔAsp = Asp - Ablk ΔAstd = Astd - Ablk			

IV. Calculations of LPS Activity

1. Standard Curve Plotting:

Plot the standard curve with the concentrations of the oleic acid standard solutions on the x-axis and their corresponding ΔAstd on the y-axis to obtain the linear regression equation:

$$y = kx + b$$

Substitute the ΔAsp as y into the equation to calculate the concentration x (μmol/mL).

2. LPS Activity Calculation

(1) Calculated by protein concentration:

Definition: One unit of enzyme activity is defined as the amount of enzyme in 1 mg of protein that hydrolyzes olive oil to produce 1μmol of fatty acid per minute at 37°C.

$$LPS\ Activity(U/mg\ Prot) = x \times Vsp \div (Cpr \times Vsp) \div T = 0.1 \times x \div Cpr$$

(2) Calculated by sample mass:

Definition: One unit of enzyme activity is defined as the amount of enzyme in 1g of tissue that hydrolyzes olive oil to produce 1μmol of fatty acid per minute at 37°C.

$$LPS\ Activity(U/g\ mass) = x \times Vsp \div (W \times Vsp \div Vex) \div T = 0.1 \times x \div W$$

(3) Calculated by serum:

Definition: One unit of enzyme activity is defined as the amount of enzyme in 1mL of serum that hydrolyzes olive oil to produce 1μmol of fatty acid per minute at 37°C.

$$LPS\ Activity(U/mL\ serum) = x \div T = 0.1 \times x$$

Note:

Vsp: Volume of the supernatant added into the reaction system = 0.2 mL;

T: Catalytic reaction time = 10min;

Cpr: Supernatant protein concentration, mg/mL(needs to be determined separately);

W: Sample mass, g;

Vex: Volume of the extraction liquid = 1mL

Precautions

1. For protein quantification, it is recommended to use the BCA Protein Quantification Kit (C0050) manufactured by TargetMol.
2. Keep away from fire sources during the experiment.
3. When the absorbance exceeds 1, it is recommended to dilute the sample before measurement.
4. This product is intended solely for scientific research use by professional personnel. It is NOT for clinical diagnosis or treatment, NOT for food or drug use, and must NOT be stored in residential areas.
5. Toluene is toxic. For your safety and health, please wear a lab coat and disposable gloves during operation.